JC14 Rec'd PCT/PTO 22 JAN 2002

SUBSTITUTE FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
10848-017001

U S APPLICATION NO. (If Known, see 37 CFR 1 5)
10/048035

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/01674	22 May 2000	22 July 1999

TITLE OF INVENTION
METHOD FOR MARKING SOLID, LIQUID AND GASEOUS SUBSTANCES

APPLICANT(S) FOR DO/EO/US
Wolf Bertling and Hans Kosak

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of amendments to the claims under PCT Article 19 (35 U S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))

Items 11 to 16 below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    ☒ PCT International Search Report (4 pages) with nine references attached
    ☐
    ☐
    ☐
    ☐

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Vince Defante
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JC13 Rec'd PCT/PTO 22 JAN 2002

U.S. APPLICATION NO. (IF KNOWN)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/048035	PCT/DE00/01674	10848-017001

17. ☒ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)- (5)):	CALCULATIONS PTO USE ONLY	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	28 - 20 =	8	x **$18**	$144.00	
Independent Claims	3 - 3 =	0	x **$84**	$0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ **$280**	$0.00	
			TOTAL OF ABOVE CALCULATIONS =	$1,034.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$517.00	
			SUBTOTAL =	$517.00	
Processing fee of **$130** for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f))				$0.00	
			TOTAL NATIONAL FEE =	$517.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$0.00	
			TOTAL FEES ENCLOSED =	$517.00	
			Amount to be refunded:		$
			Charged:		$

a. ☒ A check in the amount of $517.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Attorney's Docket No.: 10848-017001 / 412018GA-1p

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Wolf Bertling et al.	Art Unit	: Unknown
Serial No.	:	Examiner	: Unknown
Filed	:		
Title	: Method For Marking Solid, Liquid and Gaseous Substances		

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the Specification:

Please add the following paragraph on page 1 of the application after the title:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a National Stage application under 35 U.S.C. §371 and claims benefit under 35 U.S.C. §119(a) of International Application No. PCT/DE00/01674 having an International Filing Date of May 22, 2000, which claims benefit of DE 199 34 573.2 filed on July 22, 1999.--

Please delete the paragraph on page 2, lines 27-29.

In the Claims:

Please amend claims 1 and 3-21 as follows. A complete set of pending claims is shown.

1. (Amended) A method for labeling and identifying solid, liquid and gaseous substances (S1-n), comprising the steps of:

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Vince Defante
Typed or Printed Name of Person Signing Certificate

selecting at least one nucleic acid molecule from a first group of predefined nucleic acid molecules (N1-n), wherein each of the predefined nucleic acid molecules comprises an identification sequence section (IDS1-n),

contacting the substance (S1-n) with at least one predefined nucleic acid molecule (N1-n),

providing a second group of nucleic acid molecules (N'1-n), wherein each nucleic acid molecule of the second group of nucleic acid molecules comprises a detection sequence section (IDP1-n) complementary to one of the identification sequence sections (IDS1-n),

contacting the substance (S1-n) with the nucleic acid molecules (N'1-n) provided from the second group under predefined hybridization conditions; and

detecting hybridization.

2. The method as claimed in claim 1, wherein the identification sequence section (IDS1-n) is located between two primer binding sequence sections (PBS1, PBS2).

3. (Amended) The method as claimed in claim 2, wherein said identification sequence section (IDS1-n) comprises two identification sequence sections (IDS-A, IDS-B).

4. (Amended) The method as claimed in claim 3, wherein the identification sequence sections (IDS-A, IDS-B) are complementary to one another.

5. (Amended) The method as claimed in claim 2, wherein the primer binding sequence sections (PBS1, PBS2) have the same melting point.

6. (Amended) The method as claimed in claim 1, wherein the nucleic acid molecules (N1-n) are amplified.

7. (Amended) The method as claimed in claim 1, wherein the predefined nucleic acid molecules (N1-n) are linked on at least one end to an agent which counteracts degradation caused by exonuclease.

8. (Amended) The method as claimed in claim 1, wherein the predefined nucleic acid molecule (N1-n) is provided with a coupling group (A, B, C, D-Z).

9. (Amended) The method as claimed in claim 8, wherein the coupling group (A, B, C, D-Z) is selected from the group consisting of: a biotin group, an amino group, a thiol group, and a hapten.

10. (Amended) The method as claimed in claim 1, wherein a molecule carrying a fluorophoric group (F11-n) is bound to the predefined nucleic acid molecule (N1-n).

11. (Amended) The method as claimed in claim 8, wherein the coupling group (A, B, C, D-Z) is labeled with a fluorophoric group.

12. (Amended) The method as claimed in claim 1 wherein the predefined nucleic acid molecules (N1-n) are bound to the substance (S1-n) and wherein the substance (S1-n) is selected from the group consisting of antibodies, lectins, receptors, nucleotide sequences, PNA sequences, peptides, proteins, sugars, and ligands.

13. (Amended) The method as claimed in claim1, wherein the predefined nucleic acid molecules (N1-n) are bound to particles (P) or are included therein.

14. (Amended) The method as claimed in claim 13, wherein the particles (P) are from 30 nm to 3 mm in size.

15. (Amended) The method as claimed in claim 13, wherein the particles (P) are silica, polystyrene, polyvinyl chloride, polyethylene, nylon or glass milk particles.

16. (Amended) The method as claimed in claim 13, wherein the particles (P) are selected from the group consisting of a viral capsid and a virus-like particle.

17. (Amended) The method as claimed in claim 1, wherein each of the second group of nucleic acid molecules (N'1-n) is bound to a predefined site on a solid surface.

18. (Amended) The method as claimed in claim 1, wherein hybridization of an identification sequence section (IDS1-n) with a complementary detection sequence section (IDP1-n) is detected by means of fluorescence.

19. (Amended) The method as claimed in claim 1, wherein at least two predefined nucleic acid molecules (N1-n) are added to the substance (S1-n) as a label.

20. (Amended) The method as claimed in claim 1, wherein the predefined nucleic acid molecules (N1-n) and/or the second group of nucleic acid molecules (N'1-n) are prepared synthetically.

21. (Amended) The method as claimed in claim 1, wherein the first group of predefined nucleic acid molecules (N1-n) and the second group of nucleic acid molecules (N'1-n) comprise nucleic acid analogs.

Please add the following new claims:.

22. The method as claimed in claim 21, wherein the nucleic acid analogs are selected from the group consisting of PTO and PNA.

23. The method of claim 17, wherein the solid surface is selected from the group consisting of a chip, a microtiter plate, and film.

24. The method of claim 6, wherein said amplification is by PCR.

25. The method of claim 24, wherein said PCR uses fluorescently-labelled primers.

26. The method of claim 3, wherein said identification sequence sections (IDS-A, IDS-B) comprise primer binding sequence sections (PBS1, PBS2).

27. A method for identifying solid, liquid and gaseous substances (S1-n), said substance having been labeled with at least one nucleic acid molecule selected from a first group of predefined nucleic acid molecules (N1-n), wherein each of the predefined nucleic acid molecules comprises an identification sequence section (IDS1-n), comprising the steps of:

providing a second group of nucleic acid molecules (N'1-n), wherein each of the nucleic acid molecules of the second group of nucleic acid molecules comprises a detection sequence section (IDP1-n) complementary to one of the identification sequence sections (IDS1-n),

contacting the substance (S1-n) with the nucleic acid molecules (N'1-n) provided from the second group under predefined hybridization conditions; and

detecting hybridization.

28. A solid, liquid or gaseous substance (S1-n) marked with at least one nucleic acid molecule from a first group of predefined nucleic acid molecules (N1-n), wherein the article or substance has been marked by:

providing the article or substance to be marked with at least one nucleic acid molecule from a first group of predefined nucleic acid molecules (N1-n),

wherein each of the predefined nucleic acid molecules comprises an identification sequence section (IDS1-n),

wherein for identification of the predefined nucleic acid molecule, there is provided a second group of nucleic acid molecules (N'1-n), wherein each of the second group of nucleic acid molecules comprises a detection sequence section (IDP1-n) complementary to one of the identification sequence sections (IDS1-n).

In the Abstract:

Please add the Abstract on the attached page to the specification after the claims.

REMARKS

Applicants respectfully request entry of the amendments and remarks submitted herein. Claims 3-21 have been amended to remove multiple claim dependencies, and new claims 22-28 have been added. Support for the claim amendments and new claims 22-28 can be found in the originally filed claims and throughout the specification. Therefore, claims 1-28 are currently pending. Attached is a marked-up version of the changes being made by the current amendments. Reconsideration of the pending application is respectfully requested.

In addition, Applicants have amended the specification to include a paragraph describing related applications and claiming the benefit of priority to such applications, to remove the paragraph on page 2 that refers to claim numbers, and to add an Abstract. The attached Abstract is the Abstract that was published with the PCT application. Therefore, Applicants submit that there is no new matter introduced by these amendments.

CONCLUSION

Applicants ask that claims 1-28 be examined. The enclosed filing fee takes into account claims added by this Preliminary Amendment. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: January 22, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

A paragraph describing related applications has been added to page 1 after the title.

The paragraph on page 2, lines 27-29 has been deleted.

In the Claims:

Claims 1 and 3-21 have been amended as follows:

1. (Amended) A method for labeling and identifying solid, liquid and gaseous substances (S1-n), comprising the steps of:

[wherein labeling is carried out by] selecting at least one nucleic acid molecule [sequence] from a first group of predefined nucleic acid molecules [sequences] (N1-n), wherein each of the predefined nucleic acid molecules comprises [having in each case] an identification sequence section (IDS1-n) [and adding it to the substance (S1-n)],

contacting the substance (S1-n) with at least one predefined nucleic acid molecule (N1-n),

providing [wherein] a second group of [further] nucleic acid molecules [sequences] (N'1-n), wherein each nucleic acid molecule of the second group of nucleic acid molecules comprises [which have in each case] a detection sequence section (IDP1-n) complementary to one of the identification sequence sections (IDS1-n), [is provided for identification,

wherein first melting points of hybrids formed from the identification sequence sections (IDS1-n) together with the detection sequence sections (IDP1-n) complementary thereto differ by not more the 5°C from one another and

second melting points of not completely complementary hybrids from the identification sequence sections (IDS1-n) and detection sequence sections (IDP1-n) are more than 5°C lower than the lowest of the first melting points and

wherein identification is carried out by] contacting the [nucleic acid sequence(s) (N1-n) selected from the first group] substance (S1-n) with the [further] nucleic acid molecules [sequences] (N'1-n) provided from [of] the second group under predefined hybridization conditions; and

detecting hybridization.

3. (Amended) The method as claimed in claim [1 or] 2, wherein said identification sequence section (IDS1-n) comprises two identification sequence sections (IDS-A, IDS-B) [in each case two nucleic acid sequences (N1-n) have a part section (IDS-A, IDS-B) or a common identification sequence section (IDS1-n) at their 5' end and a primer binding sequence section is bound to said part section (IDS-A, IDS-B)].

4. (Amended) The method as claimed in claim 3, wherein the [part sections] identification sequence sections (IDS-A, IDS-B) are [partly] complementary to one another.

5. (Amended) The method as claimed in claim 2 [any of the preceding claims], wherein the primer binding sequence sections (PBS1, PBS2) have the same melting point.

6. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein the nucleic acid molecules [sequences] (N1-n) are amplified[, preferably by means of PCR and by using fluorescent primers].

7. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein the predefined nucleic acid molecules [sequences] (N1-n) are linked on at least one end to an agent which counteracts degradation caused by exonuclease.

8. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein the predefined nucleic acid molecule [sequence] (N1-n) is provided with a coupling group (A, B, C, D-Z).

9. (Amended) The method as claimed in claim 8 [any of the preceding claims], wherein the coupling group (A, B, C, D-Z) is selected from the [following] group consisting of [[sic]]: a biotin group, an amino group, a thiol group, and a [or] hapten.

10. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein a molecule carrying a fluorophoric group (F11-n) is bound to the predefined nucleic acid molecule [sequence] (N1-n).

11. (Amended) The method as claimed in claim 8 [any of the preceding claims], wherein the coupling group (A, B, C, D-Z) is labeled with a fluorophoric group.

12. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein the predefined nucleic acid molecules [sequences] (N1-n) are bound to the substance (S1-n) and wherein the substance (S1-n) [used] is selected from the group consisting of [one of the

following agents:] antibodies, lectins, receptors, nucleotide sequences, PNA sequences, peptides, proteins, sugars, and ligands.

13. (Amended) The method as claimed in claim1 [any of the preceding claims], wherein the predefined nucleic acid molecules [sequences] (N1-n) are bound to particles (P) or are included therein.

14. (Amended) The method as claimed in claim 13 [any of the preceding claims], wherein the particles (P) are from 30 nm to 3 mm in size.

15. (Amended) The method as claimed in claim 13 [any of the preceding claims], wherein the particles (P) are silica, polystyrene, polyvinyl chloride, polyethylene, nylon or glass milk particles.

16. (Amended) The method as claimed in claim 13 [any of the preceding claims], wherein the particles (P) [is] are selected from the group consisting of a viral capsid [or] and a virus-like particle.

17. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein each of the [further] second group of nucleic acid molecules [sequences] (N'1-n) is bound to a predefined site on a solid surface[, preferably on a chip, a microtiter plate or film].

18. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein hybridization of an identification sequence section (IDS1-n) with a complementary detection sequence section (IDP1-n) is detected by means of fluorescence.

19. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein at least two predefined nucleic acid molecules [sequences] (N1-n) are added to the substance (S1-n) as a label.

20. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein the predefined nucleic acid molecules [sequences] (N1-n) and/or the [further] second group of nucleic acid molecules [sequences] (N'1-n) are prepared synthetically.

21. (Amended) The method as claimed in claim 1 [any of the preceding claims], wherein the first group of predefined nucleic acid molecules (N1-n) and the second group of nucleic acid molecules (N'1-n) comprise nucleic acid analogs [chimeras of nucleic acids and nucleic acid analogs, such as PTO or PNA, are used instead of the nucleic acid sequences or the further nucleic acid sequences].

New claims 22-28 have been added.

In the Abstract:

The Abstract on the attached page has been added to the application.

ABSTRACT OF THE DISCLOSURE

The invention relates to a method for marking and identifying solid, liquid and gaseous substances (S1-n). In order to carry out said marking, at least one nucleic acid sequence is selected from a first group of predefined nucleic acid sequences (N1-n) respectively possessing an identification sequence section (IDS1-n) and is added to the substance (S1-n). In order to carry out identification, a second group of other nucleic acid sequences (N'1-n) is provided, whereby said nucleic acid sequences respectively posses a detection sequence section (IDP1-n) which is complementary to one of the identification sequence sections (IDS1-n). First melting points of the hybrids which are formed from the identification sequences (IDS1-n) with the complementary detection sequence sections (IDP1-n) differ by a maximum of 5°C and second melting points of hybrids which are not fully complementary formed from the identification sequence sections (IDS1-n) and detection sequences (IDP1-n), are lower by more than 5°C than the lowest first melting point. For identification purposes, the nucleic acid sequences selected from the first group (N1-n) are brought into contact with the other nucleic acid sequences (N'1-n) from the second group in predefined hybridization conditions and said hybridization is detected.

Method for labeling solid, liquid and gaseous substances

The invention relates to a method for labeling solid, liquid and gaseous substances.

According to the prior art, various methods are known, which utilize nucleic acid sequences for labeling.

WO 90/14441 discloses a method in which a predefined specific nucleic acid sequence is amplified and subsequently identified.- This cannot be used for complex labeling in the style of a code.

WO 91/17265 describes a method for labeling a material with microtraces of DNA. The DNA which is present in the smallest amounts is amplified by a PCR. The DNA is identified by sequencing. Detection of the DNA employed for labeling is time-consuming and complicated.

US 5,866,336 discloses methods in which a formation of intra- or intermolecular hybrids is detected by energy transfer between donor and acceptor molecules.

WO 94/04918 utilizes a nucleic acid bound to a particle for labeling a liquid. The nucleic acid is amplified and identified, for example, by means of a radioactive label.

WO 95/02702 discloses a method which uses various nucleic acids in combination with various particles. This can be used to generate a complex labeling code. The nucleic acids are identified by means of PCR amplification, where appropriate in combination with sequencing. In order to identify the code, furthermore identification of the particles and combination with the information obtained from the identifications are required.

US 5,656,731 describes a labeling of antibodies by means of nucleic acids. The presence of the sought-after antibody is carried out [sic], after a preceding selection process, on the basis of detecting the nucleic acid. For this purpose, the nucleic acid is amplified.

US 5,708,153 describes a labeling of polymeric substances. In this connection, simultaneously with the synthesis of the polymeric substance, a nucleic acid monitoring the synthesis is synthesized. The composition of the polymeric substance is identified by amplifying and sequencing the nucleic acid.

The methods known are costly and time-consuming. As far as a sequencing reaction is required for identifying the label, utilization of a complex labeling code or identification of a label from a plurality of labels is possible only with extreme difficulties.

It is an object of the invention to indicate a method which can be used to label unambiguously a plurality of substances and to identify them rapidly and inexpensively.

This object is achieved by the features of claim 1. Expedient embodiments result from the features of claims 2 to 21.

In accordance with the invention a method for labeling and identifying solid, liquid and gaseous substances is provided for wherein labeling is carried out by selecting at least one nucleic acid sequence from a first group of predefined nucleic acid sequences having in each case a labeling [sic] sequence section and adding it to the substance,

wherein a second group of further nucleic acid sequences which have in each case a detection sequence section complementary to one of the identification sequence sections is provided for identification,

wherein first melting points of hybrids formed from the identification sequence sections together with the detection sequence sections complementary thereto differ by not more than 5°C from one another and

second melting points of not completely complementary hybrids from the identification sequence sections and detection sequence sections are more than 5°C lower than the lowest of the first melting points and

wherein identification is carried out by contacting the nucleic acid sequence(s) selected from the first group with the further nucleic acid sequences of the second group under predefined hybridization conditions and detecting hybridization.

The method can be carried out easily, rapidly and inexpensively. It is possible to amplify and identify all identification sections used for labeling in a single reaction mixture.

Nucleic acid sequences here mean both single- and double-stranded sequences which essentially consist of nucleic acids.

According to one embodiment feature, the identification sequence section is located between two primer binding sequence sections. - In this case, the nucleic acid sequence may be single-stranded. Amplification is readily possible, for example, by means of the polymerase chain reaction (PCR).

According to a further embodiment, in each case two nucleic acid sequences have a part section of a common

identification sequence section at their 5' end and a primer binding sequence section is bound to said part section. In this embodiment the identification sequence section is initially not present but is generated only during the amplification reaction. Advantageously, the part sections are in this connection partly complementary to one another. - This increases the reliability of the labeling.

Expediently, the primer binding sequence sections have the same melting point. This makes possible a simultaneous amplification reaction of the nucleic acid sequences in a single reaction mixture.

The nucleic acid sequences may be amplified preferably by means of PCR and by using fluorescent primers. In order to improve the stability of the label, it is advantageously provided for the nucleic acid sequences to be linked on at least one end to an agent which counteracts degradation caused by exonuclease.

Complex labels may be generated by providing the nucleic acid sequences with a specific coupling group. The coupling group may be selected from the following group [sic]: biotin group, amino group, thiol group or hapten. By means of the coupling group the nucleic acid sequence provided therewith can be bound specifically and/or, where appropriate, also be identified.

Further primer binding sequence sections may also be used for identification. Each primer binding sequence section can be identified unambiguously on the basis of a specific fluorophoric group bound thereto.

Identification can be made easier further by a molecule carrying a fluorophoric group, which is bound to the nucleic acid sequence.

In particular when using identical identification sequence sections, it is possible also to use further primer binding sequence sections for identification. Each of the primer binding sequence sections can be identified unambiguously on the basis of a specific fluorophoric group bound thereto.

It has proved to be expedient to bind the nucleic acid sequence to the substance and to use as substance one of the following agents: antibodies, lectins, receptors, nucleotide sequences, PNA sequences, peptides, proteins, sugars, ligands. It is regarded as particularly advantageous to bind the nucleic acid sequences to particles or to include them therein. The particles may be from 30 nm to 3 mm in size. They are advantageously silica, polystyrene, polyvinyl chloride, polyethylene, nylon or glass milk particles. However, the particle may also be a viral capsid or a virus-like particle. - The use of nucleic acid sequence-carrying particles is particularly advantageous, because it is possible, owing to the size of the particles, to sort and isolate them, for example, in a particle sorter. The particle may be bound as label carrier to the substance to be labeled.

It has proved advantageous for identification that each of the further nucleic acid sequences is bound to a predefined site of a solid surface, preferably on a chip, a microtiter plate or film. This makes it possible to decode complex labeling codes or a multiplicity of codes in a single process step.

Hybridization of an identification sequence section with a complementary detection sequence section can be detected by means of fluorescence. The proposed identification can be detected particularly easily.

According to a further embodiment, it is provided for at least two nucleic acid sequences to be added to the

substance as a label. It is thereby possible to provide a complex code by using a small number of different identification sequence sections.

The nucleic acid sequences and/or the further nucleic acid sequences are preferably prepared synthetically.

It is also possible to use chimeras of nucleic acids and nucleic acid analogs, such as PTO or PNA, instead of the nucleic acid sequences or the further nucleic acid sequences. Such chimeras have increased stability against enzymatic degradation.

On the basis of the drawing [sic], exemplary embodiments of the invention are illustrated in more detail below. The figures show:

Figs. 1a - e amplification of a first nucleic acid sequence,

Figs. 2a - e amplification of two second nucleic acid sequences,

Figs. 3a - d identification of a first nucleic acid sequence by means of molecular beacons,

Fig. 4 labeling of an identification sequence section,

Fig. 5a nucleic acid sequences labeled with fluorophoric groups,

Figs. 5b, c a detector with the further nucleic acid sequences,

Figs. 6a - d selection and identification of third nucleic acid sequences,

Figs. 7a - f preparation of a complex code,

Figs. 8a - d preparation of labeling particles,

Figs. 9a - d labeling of molecules with labeling particles according to Fig. 8 and

Fig. 10 selection and identification of substances labeled with labeling particles.

Figs. 1a - e depict diagrammatically a first nucleic acid sequence N(I)1 and amplification thereof. The first nucleic acid sequence N(I)1 has at its 3′ end a first primer binding sequence section PBS1 and at its 5′ end a second primer binding sequence section PBS′2 complementary to a second primer binding sequence section PBS2 (not shown here). An identification sequence section IDS is located between the first PBS1 and the second complementary primer binding sequence section PBS′2.

For amplification, the first nucleic acid sequence N(I)1 is contacted with a first primer P1 and a second primer P2. The first P1 and the second primer P2 hybridize with the first primer binding sequence section PBS1 and the second primer binding sequence section PBS2, respectively, which are complementary thereto. The first P1 and the second primer P2 are extended by polymerase; an identification sequence section IDS′ complementary to the identification sequence section IDS is formed (Fig. 1c). The second primer P2 then binds to the complementary nucleic acid sequence N′(I)1 (Fig. 1d). Then a double-stranded DNA which contains the identification sequence section IDS is formed by the polymerase (Fig. 1e).

In the exemplary embodiment depicted in Figs. 2a-e the identification sequence section IDS is initially not present. A second nucleic acid sequence N(II)1 has at

its 5′ end the first primer binding sequence section PBS1. A part section IDS-A of the common identification sequence section IDS is bound thereto. A further second nucleic acid sequence N(II)2 has at its 3′ end the second primer binding sequence section PBS2. A second part section IDS-B of the identification sequence section IDS is bound thereto. The first part section IDS-A and the second part section IDS-B are in sections complementary to one another.

Figs. 2b - e depict the amplification. The first primer P1 and the second primer P2 bind to the primer sequence sections PBS1 and PBS2, respectively, which are in each case complementary thereto. The complementary sequences of the first IDS-A and of the second part section IDS-B are synthesized by polymerase (Fig. 2c). These synthesis products may hybridize in further cycles at their 3′ ends (Fig. 2d) and may be extended (Fig. 2e). The product formed is a nucleic acid sequence which has the complete identification sequence section IDS.

Advantageously, it is possible in this method variant to use nucleic acid analogs such as, for example, PNA or PTO in the region of the first PBS1 and second primer binding sequence section PBS2. Such nucleic acid analogs have increased stability against enzymatic degradation. The stability can also be further increased by coupling the 5′ end of the nucleic acid sequence to an agent which prevents 5′ exonuclease degradation. Suitable agents for this purpose are, for example, PNA or PTO.

The primer binding sequence sections PBS1, PBS2 are advantageously chosen such that the amplification reaction can be carried out within a narrow temperature range. For this purpose, the primer binding sequence sections PBS1 and PBS2 are chosen such that their melting points differ by not more than 5° Celsius from one another. In order to increase the specificity of

the identification reaction, it is advantageous that the melting point of the primer binding sequence sections PBS1 or PBS2 in a formation of not completely complementary hybrids is more than 5° Celsius below the lowest melting point of a completely complementary hybrid. This renders impossible the formation of unspecific hybrids during amplification.

The labels are identified by hybridizing the identification sequence section IDS with a detection sequence section completely complementary thereto. In order to increase the specificity of the identification reaction, it is provided for all identification sequence sections IDS together with the detection sequence sections IDP complementary thereto to have first melting points which preferably differ by not more than 5° Celsius from one another. To further increase the specificity, it is provided for to be each melting point of an incomplete hybrid with an identification sequence section IDS to be more than 5° Celsius below the lowest melting point of completely complementary hybrids.

In order to further simplify carrying out the method, it is additionally provided for the melting points of the primer binding sequence sections PBS1, PBS2 and the identification sequence sections IDS to be essentially identical.

According to another variant of the method, a first nucleic acid sequence N(I)1 is duplicated in an amplification reaction (Figs. 3a and b). The reaction mixture additionally contains molecular beacons having detection sequence sections IDP1-n complementary to the identification sequence sections. The molecular beacons MB have the shape of a hairpin loop. A fluorophoric group F11, F12, F13 and, arranged opposite, a quencher Q1, Q2, Q3 are located in the end regions of the molecular beacons.

As soon as a sufficient number of first nucleic acid sequences N(I)1-n has been prepared by amplification, the identification sequence section IDS1-n hybridizes specifically with the detection sequence section IDP1-n complementary thereto. In this connection, the spatial relationship between the quencher Q1, Q2, Q3 and the fluorophoric group F11, F12, F13 is terminated. A specific fluorescence can be detected (Fig. 3d).

Using various fluorophores F11, F12, F13 in the molecular beacons MB makes it possible to distinguish various identification sequence sections IDS1-n.

In the variant of the method depicted in Figs. 4a-c, amplification is carried out using a first primer P1 which is labeled with a fluorophoric group F11. When using nucleic acid sequences having an identical identification sequence section IDS, it is possible to distinguish said nucleic acid sequences by using different primer binding sequence sections which are labeled in each case with specific fluorophoric groups.

The identification sequence section IDS is identified by contacting the nucleic acid sequence, prepared in the amplification using the fluorophoric group F11, with a complementary detection nucleotide sequence IDP which is bound at a predefined site of a solid surface. The fluorescence which then appears at the predefined site may be recorded by means of a conventional detection apparatus (see Figs. 5a - c).

According to another variant of the method, the nucleic acid sequences N1-n are linked to coupling groups which makes it possible to bind to further substances. Said coupling groups include biotin, amino linkers, thiol group or haptens, such as digoxigenin. Using said coupling groups, the nucleic acid sequence N1-n may be

bound to molecules to be labeled specifically and/or may be labeled.

It is possible to label unambiguously the different antibodies with the inventive nucleic acid sequence N1-n by means of the coupling groups generally denoted CG. The binding may be mediated via a protein A/streptavidin fusion protein. This protein binds the constant antibody region and mediates affinity to biotin. Biotinylated nucleic acid sequences bind to such antibodies, thus making selection possible. Figs. 6a - d depict such a selection. Antibodies A, B, C, D and Z have been labeled with the nucleic acid sequences N1, N2, N4, N49 and Nn. The antibodies A, B, C, D and Z are contacted with a matrix to which various antigens A', C', X', D' and Y' have been attached. The antibodies A, C, D, whose antigens A', C' and D' have been attached to the matrix, are bound specifically (Fig. 6b). After removing the antibodies B, Z which have not bound to the matrix by washing, the nucleic acid sequences N1, N4 and N49 may be amplified using labeled primers (Fig. 6c and Fig. 5a, respectively). The amplified nucleic acid sequences N1, N4, N49 are contacted with a detector surface to which the further nucleic acid sequences N1-n containing the detection sequence sections IDP are bound. In this connection, each further nucleic acid sequence N'1-n occupies a particular predefined position on the detector surface (see Fig. 6d).

Figs. 7a - f depict the preparation and identification of a complex code in the form of a flow chart. 50 different nucleic acid sequences are used. The 50 nucleic acid sequences are divided into 5 groups of 10 nucleic acid sequences each (Fig. 7b). A numeral from 0 to 9 is assigned to each nucleic acid sequence of a group (Fig. 7c). One figure of a 5-figure number is assigned to each group of nucleic acid sequences (Fig. 7d).

The code is prepared by taking exactly one nucleic acid sequence from each group of the 5 groups and using said nucleic acid sequence for labeling (Fig. 7e). Each mixture of the 5 nucleic acid sequences taken thus defines a number between 0 and 99999. The numerical value is identified by identification of the 5 nucleic acid sequences (Fig. 7f).

According to another embodiment of the method, the nucleic acid sequences N1-n may be bound to particles P. Binding takes place preferably via activated or activatable groups. These groups include biotin, aminolinkers, thiol groups or haptens such as digoxigenin. Particles P which may be used are polystyrene, silica, polyvinyl chloride, polyethylene, nylon or glass milk particles. It is also possible to make use of particles consisting of virus coats or virus-like particles. The particles P may also be prepared from agents which complex with DNA, such as, for example, polylysine or DNA-binding proteins.

The particles P may be labeled with a variety of nucleic acid sequences N1-n. A particle P may therefore carry a numerical code according to the abovementioned example. Figs. 8a-d depict the preparation of a labeled particle P by way of example. The nucleic acid sequence N1-n carries on one of its ends a biotin group (Fig. 8a). A variety of such biotinylated nucleic acid sequences are dissolved in the same molar ratio (Fig. 8b). The solution is admixed with a predefined amount of particles P which have been coated, for example, with streptavidin (Fig. 8c). A bond between biotin and streptavidin is formed. The nucleic acid sequences N1-n are thus bound to the particle P (Fig. 8d). It is, of course, possible for a plurality of nucleic acid sequences N1-n of the same type to be bound on the particle P. This increases the reliability of the reaction during amplification.

The particles P are preferably from 1 µm to 100 µm in size. They may be fluorescent or may be fluorescently labeled with other agents via a binding reaction. Due to their size and fluorescent property, the particles P may be sorted and isolated by means of a particle sorter. This makes it possible to identify the numberings of individual particles P which are part of a mixture of several particles P.

According to another embodiment of the invention, the particles P may also have coupling groups CG which are suitable for binding to the substance to be labeled (Fig. 9a). These groups include biotin groups, aminolinker groups or thiol groups. They may be attached, for example, at the end of the nucleic acid sequence N1-n. The coupling groups CG are bound to the particle preferably via spacer molecules L (Fig. 9b).

Fig. 9c depicts a particle P with free coupling groups CG. Fig. 9d depicts a particle P in which the substance S is bound to the coupling groups CG.

The substance S1-n may be reacted, for example, with a potential receptor R (Fig. 10a). The receptor R may be labeled with a fluorophoric molecule (Fig. 10b). Particles P carrying a ligand of the receptor R are bound by the receptor R. Particles P containing the receptor R may be separated, owing to their size and fluorescence, from particles P, which have no receptor R bound, by means of a fluorescence-activated particle sorter (Fig. 10c). The bound substance may be identified on the basis of the identification sequence section IDS of the nucleic acid sequence N1-n.

List of reference symbols

N1-n	Nucleic acid sequence
N'1-n	Further nucleic acid sequence
IDS1-n	Identification sequence section
IDP1-n	Detection sequence section
IDS-A, IDS-B	Part section
PBS1	First primer binding sequence section
PBS2	Second primer binding sequence section
CG, A,B,C,D,Z	Coupling groups
L	Spacer molecule
S	Substance
R	Receptor
F11	Fluorophoric group
P	Particle

Patent claims

1. A method for labeling and identifying solid, liquid and gaseous substances (S1-n),

wherein labeling is carried out by selecting at least one nucleic acid sequence from a first group of predefined nucleic acid sequences (N1-n) having in each case an identification sequence section (IDS1-n) and adding it to the substance (S1-n),

wherein a second group of further nucleic acid sequences (N'1-n) which have in each case a detection sequence section (IDP1-n) complementary to one of the identification sequence sections (IDS1-n) is provided for identification,

wherein first melting points of hybrids formed from the identification sequence sections (IDS1-n) together with the detection sequence sections (IDP1-n) complementary thereto differ by not more than 5°C from one another and

second melting points of not completely complementary hybrids from the identification sequence sections (IDS1-n) and detection sequence sections (IDP1-n) are more than 5°C lower than the lowest of the first melting points and

wherein identification is carried out by contacting the nucleic acid sequence(s) (N1-n) selected from the first group with the further nucleic acid sequences (N'1-n) of the second group under predefined hybridization conditions and detecting hybridization.

2. The method as claimed in claim 1, wherein the identification sequence section (IDS1-n) is

located between two primer binding sequence sections (PBS1, PBS2).

3. The method as claimed in claim 1 or 2, wherein in each case two nucleic acid sequences (N1-n) have a part section (IDS-A, IDS-B) of a common identification sequence section (IDS1-n) at their 5' end and a primer binding sequence section is bound to said part section (IDS-A, IDS-B).

4. The method as claimed in claim 3, wherein the part sections (IDS-A, IDS-B) are partly complementary to one another.

5. The method as claimed in any of the preceding claims, wherein the primer binding sequence sections (PBS1, PBS2) have the same melting point.

6. The method as claimed in any of the preceding claims, wherein the nucleic acid sequences (N1-n) are amplified, preferably by means of PCR and by using fluorescent primers.

7. The method as claimed in any of the preceding claims, wherein the nucleic acid sequences (N1-n) are linked on at least one end to an agent which counteracts degradation caused by exonuclease.

8. The method as claimed in any of the preceding claims, wherein the nucleic acid sequence (N1-n) is provided with a coupling group (A, B, C, D - Z).

9. The method as claimed in any of the preceding claims, wherein the coupling group (A, B, C, D - Z) is selected from the following group [sic]: biotin group, amino group, thiol group or hapten.

10. The method as claimed in any of the preceding claims, wherein a molecule carrying a fluorophoric group (F11-n) is bound to the nucleic acid sequence (N1-n).

11. The method as claimed in any of the preceding claims, wherein the coupling group (A, B, C, D - Z) is labeled with a fluorophoric group.

12. The method as claimed in any of the preceding claims, wherein the nucleic acid sequences (N1-n) are bound to the substance (S1-n) and the substance (S1-n) used is one of the following agents: antibodies, lectins, receptors, nucleotide sequences, PNA sequences, peptides, proteins, sugars, ligands.

13. The method as claimed in any of the preceding claims, wherein the nucleic acid sequences (N1-n) are bound to particles (P) or are included therein.

14. The method as claimed in any of the preceding claims, wherein the particles (P) are from 30 nm to 3 mm in size.

15. The method as claimed in any of the preceding claims, wherein the particles (P) are silica, polystyrene, polyvinyl chloride, polyethylene, nylon or glass milk particles.

16. The method as claimed in any of the preceding claims, wherein the particle (P) is a viral capsid or a virus-like particle.

17. The method as claimed in any of the preceding claims, wherein each of the further nucleic acid sequences (N'1-n) is bound to a predefined site on

a solid surface, preferably on a chip, a microtiter plate or film.

18. The method as claimed in any of the preceding claims, wherein hybridization of an identification sequence section (IDS1-n) with a complementary detection sequence section (IDP1-n) is detected by means of fluorescence.

19. The method as claimed in any of the preceding claims, wherein at least two nucleic acid sequences (N1-n) are added to the substance (S1-n) as a label.

20. The method as claimed in any of the preceding claims, wherein the nucleic acid sequences (N1-n) and/or the further nucleic acid sequences (N'1-n) are prepared synthetically.

21. The method as claimed in any of the preceding claims, wherein chimeras of nucleic acids and nucleic acid analogs, such as PTO or PNA, are used instead of the nucleic acid sequences or the further nucleic acid sequences.

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[Fortsetzung auf der nächsten Seite]

(54) Title: METHOD FOR MARKING SOLID, LIQUID AND GASEOUS SUBSTANCES

(54) Bezeichnung: VERFAHREN ZUR MARKIERUNG VON FESTEN, FLÜSSIGEN UND GASFÖRMIGEN SUBSTANZEN

(57) **Abstract:** The invention relates to a method for marking and identifying solid, liquid and gaseous substances (S1-n). In order to carry out said marking, at least one nucleic acid sequence is selected from a first group of predefined nucleic acid sequences (N1-n) respectively possessing an identification sequence section (IDS1-n) and is added to the substance (S1-n). In order to carry out identification, a second group of other nucleic acid sequences (N'1-n) is provided, whereby said nucleic acid sequences respectively posses a detection sequence section (IDP1-n) which is complementary to one of the identification sequence sections (IDS1-n). First melting points of the hybrids which are formed from the identification sequences (IDS1-n) with the complementary detection sequence sections (IDP1-n) differ by a maximum of 5 °C and second melting points of hybrids which are not fully complementary, formed from the identification sequence sections (IDS1-n) and detection sequences (IDP1-n), are lower by more than 5 °C than the lowest first melting point. For identification purposes, the nucleic acid sequences selected from the first group (N1-n) are brought into contact with the other nucleic acid sequences (N'1-n) from the second group in predefined hybridization conditions and said hybridization is detected.

(57) **Zusammenfassung:** Die Erfindung betrifft ein Verfahren zur Markierung und Identifizierung von festen, flüssigen und gasförmigen Substanzen (S1-n), wobei zur Markierung aus einer ersten Gruppe vorgegebener jeweils einen Identifizierungssequenzabschnitt (IDS1-n) aufweisenden Nukleinsäuresequenzen (N1-n) mindestens eine ausgewählt und zur Substanz (S1-n) hinzugefügt wird, wobei zur Identifizierung eine zweite Gruppe weitere Nukleinsäuresequenzen (N'1-n) vorgesehen ist, die jeweils einen zu einem der Identifizierungssequenzabschnitte (IDS1-n) komplementären Nachweissequenzabschnitt (IDP1-n) aufweisen, wobei erste Schmelzpunkte von aus den Identifizierungssequenzabschnitten (IDS1-n) mit dem dazu komplementären Nachweissequenzabschnitten (IDP1-n) gebildeten, Hybriden sich um höchstens 5°C voneinander unterscheiden und zweite Schmelzpunkte von aus den Identifizierungssequenzabschnitten (IDS1-n) und Nachweissequenzabschnitten (IDP1-n) gebildeten nicht vollständig komplementären Hybriden um mehr als 5°C niedriger sind als der niedrigste der ersten Schmelzpunkte und wobei zur Identifizierung die aus der ersten Gruppe ausgewählte/n Nukleinsäuresequenz/en (N1-n) mit den weiteren Nukleinsäuresequenzen (N'1-n) der zweiten Gruppe unter vorgegebenen Hybridisierungsbedingungen in Kontakt gebracht und die Hybridisierung nachgewiesen wird.

WO 01/07645 A3

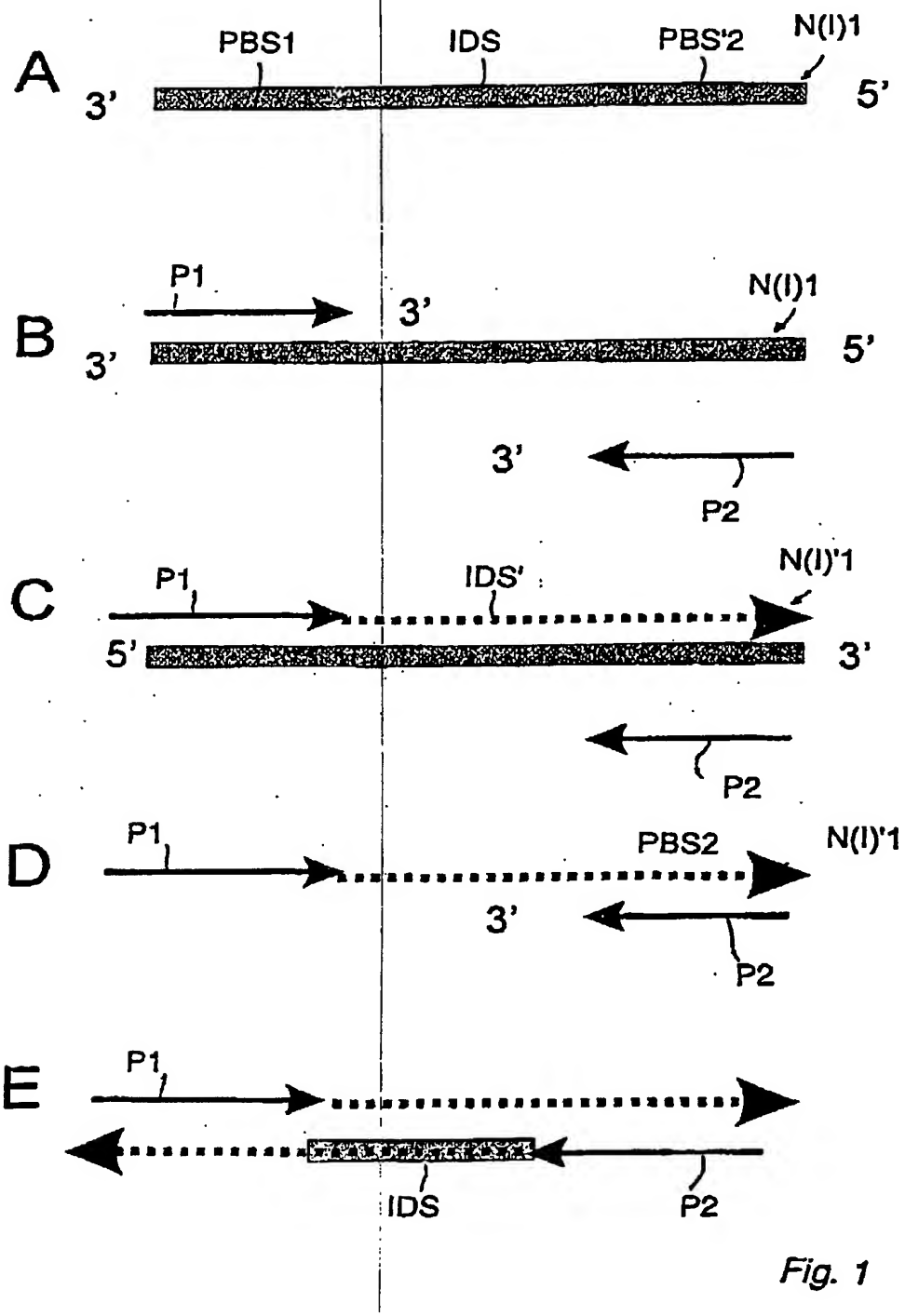

A
PBS1
IDS
PBS'2
N(I)1
3'
5'
B
P1
3'
N(I)1
3'
5'
3'
P2
C
P1
IDS'
N(I)'1
5'
3'
P2
D
P1
PBS2
N(I)'1
3'
P2
E
P1
IDS
P2

Fig. 1

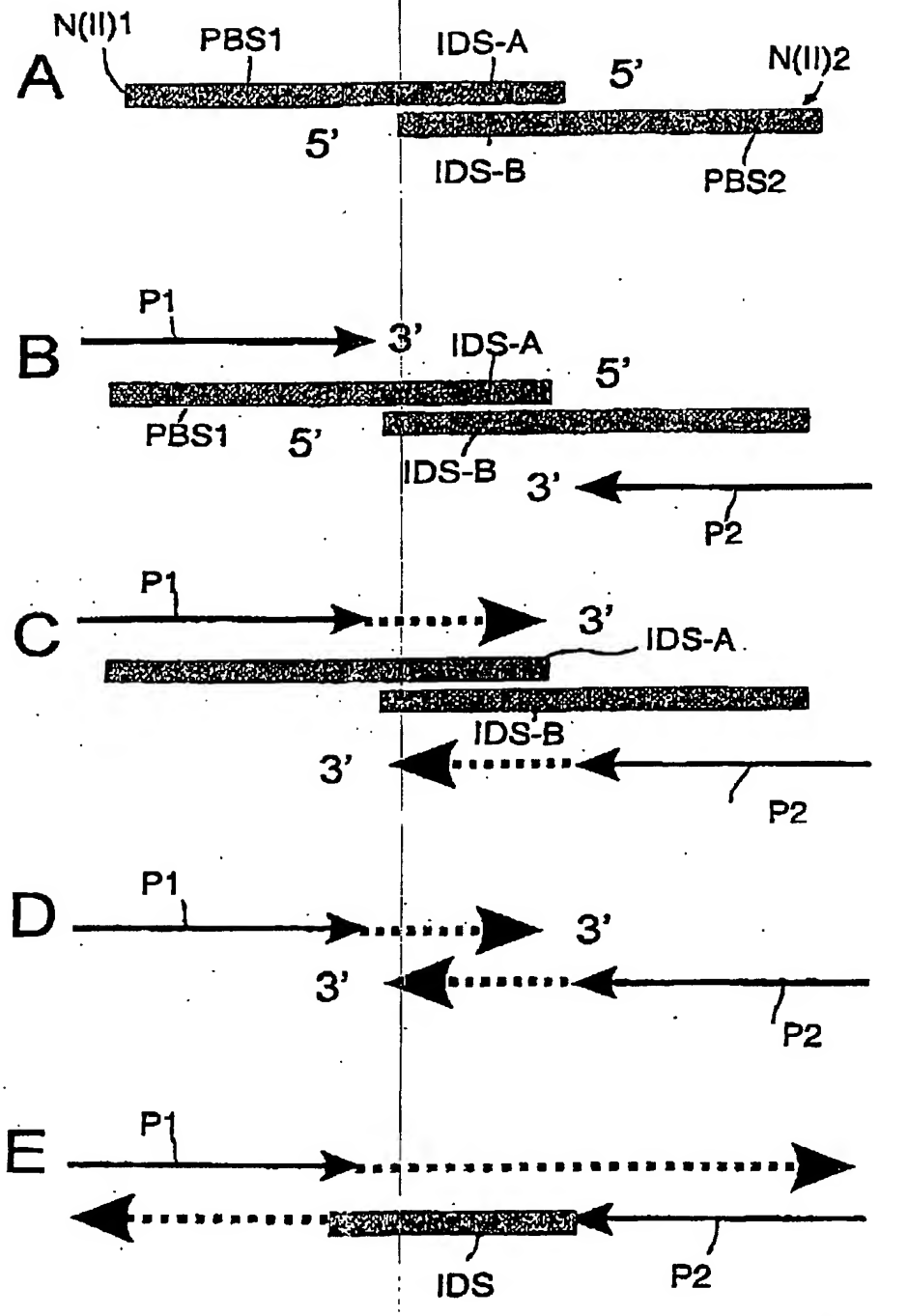

A
N(II)1
PBS1
IDS-A
5'
N(II)2
5'
IDS-B
PBS2
B
P1
3'
IDS-A
5'
PBS1
5'
IDS-B
3'
P2
C
P1
3'
IDS-A
IDS-B
3'
P2
D
P1
3'
3'
P2
E
P1
IDS
P2

Fig. 2

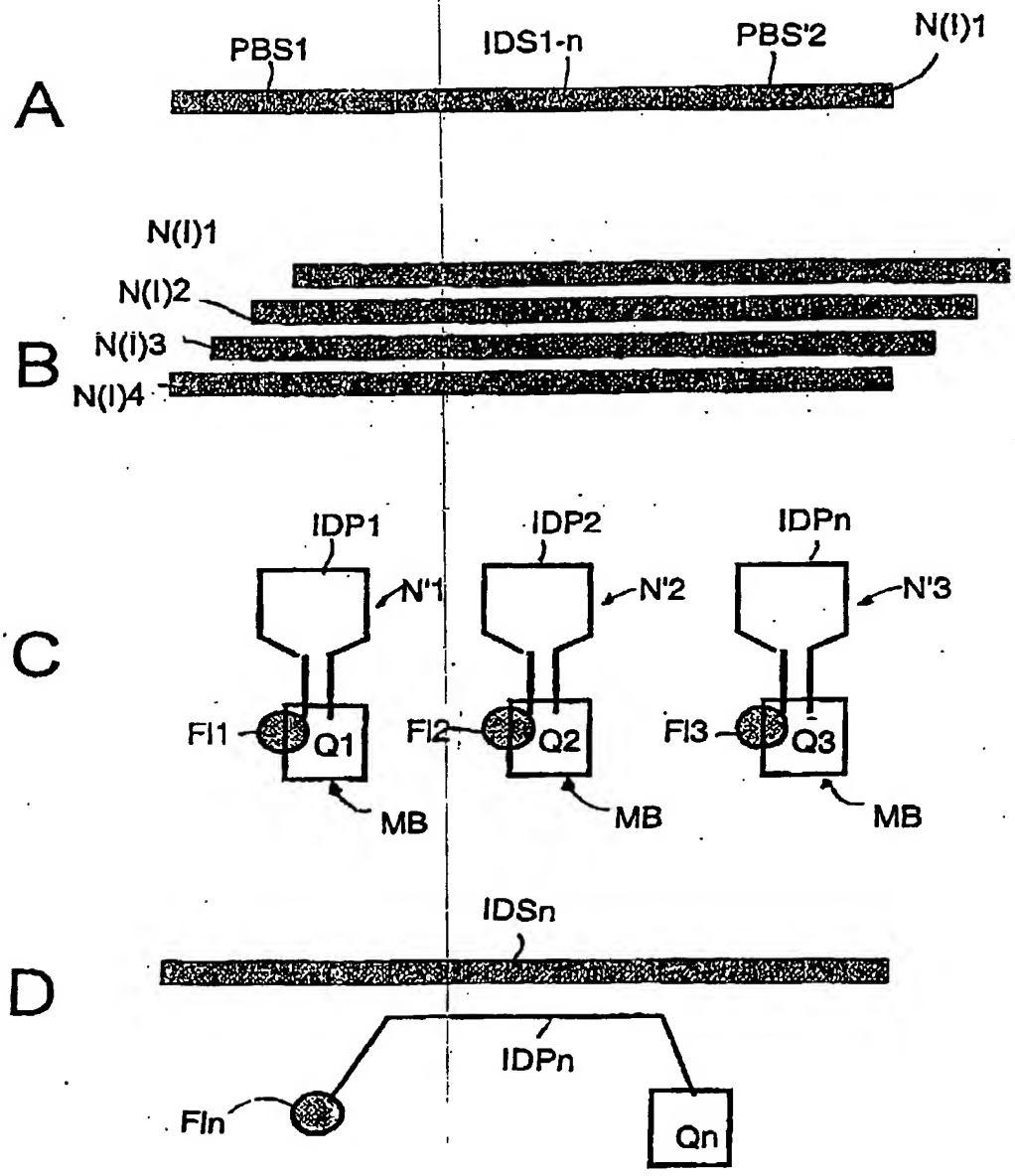

A
PBS1
IDS1-n
PBS'2
N(I)1
B
N(I)1
N(I)2
N(I)3
N(I)4
C
IDP1
IDP2
IDPn
N'1
N'2
N'3
FI1
Q1
FI2
Q2
FI3
Q3
MB
MB
MB
D
IDSn
IDPn
FIn
Qn

Fig. 3

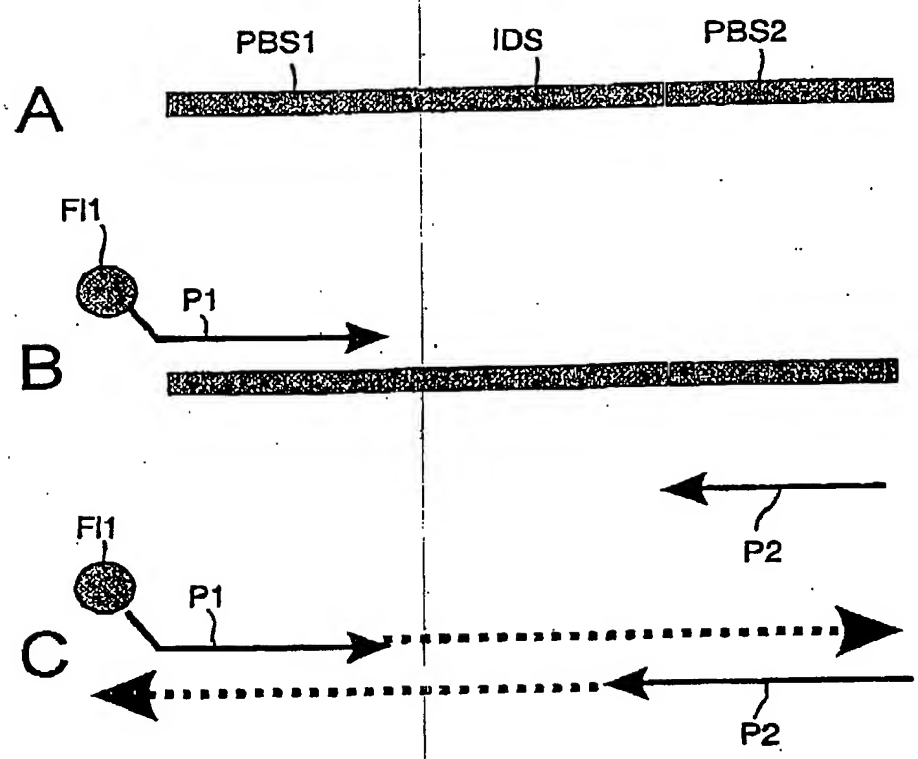
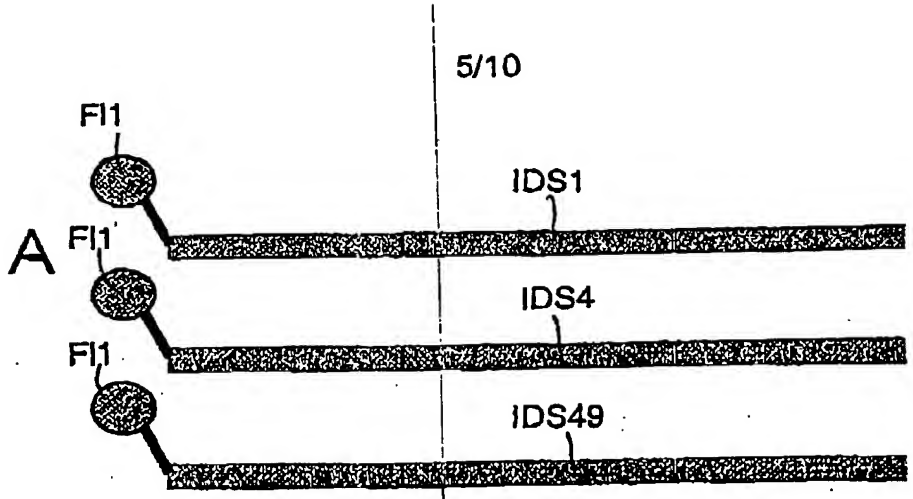
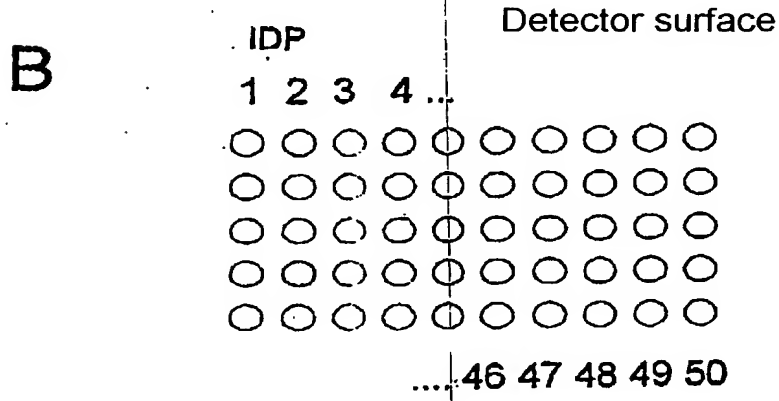


Fig. 4


A
Fl1
IDS1
Fl1
IDS4
Fl1
IDS49


B
Detector surface
IDP
1 2 3 4 ...
....46 47 48 49 50

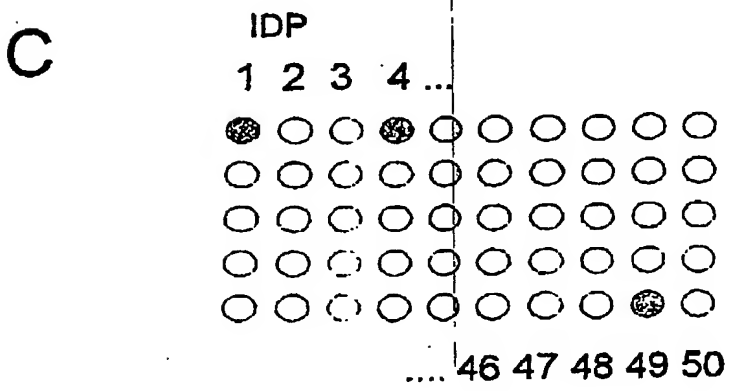
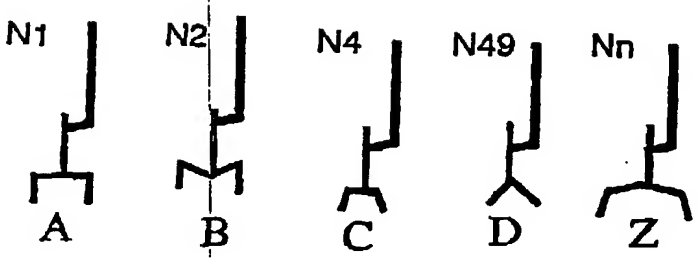
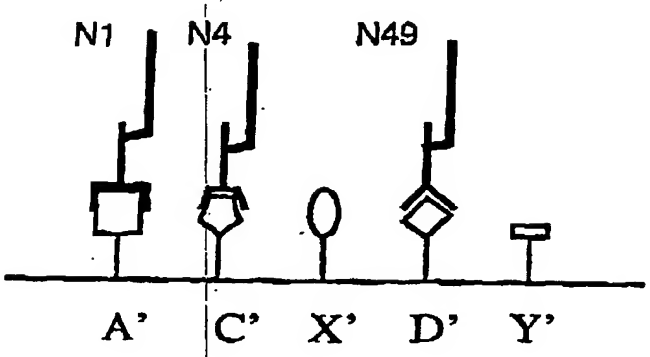

C
IDP
1 2 3 4 ...
.... 46 47 48 49 50

Fig. 5

A



B



C

Amplification using labeled primers

D

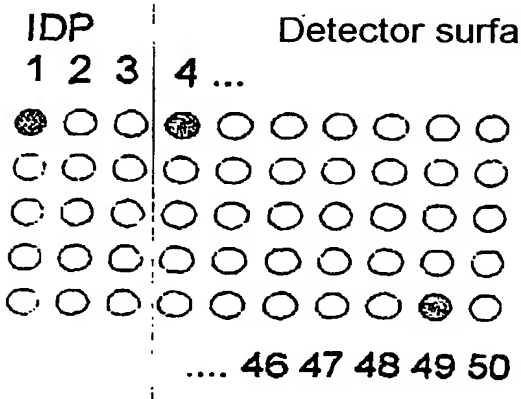


Fig. 6

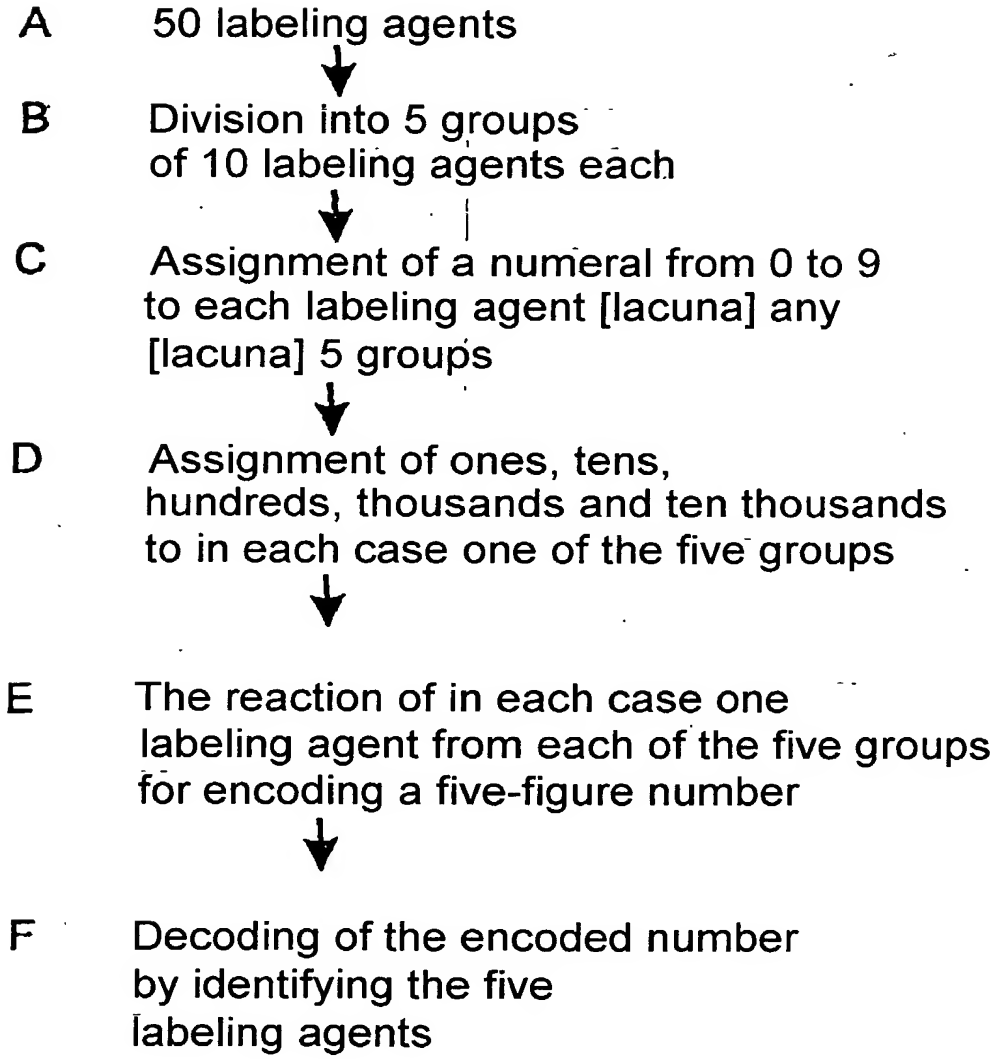

A
50 labeling agents
B
Division into 5 groups of 10 labeling agents each
C
Assignment of a numeral from 0 to 9 to each labeling agent [lacuna] any [lacuna] 5 groups
D
Assignment of ones, tens, hundreds, thousands and ten thousands to in each case one of the five groups
E
The reaction of in each case one labeling agent from each of the five groups for encoding a five-figure number
F
Decoding of the encoded number by identifying the five labeling agents

Fig. 7

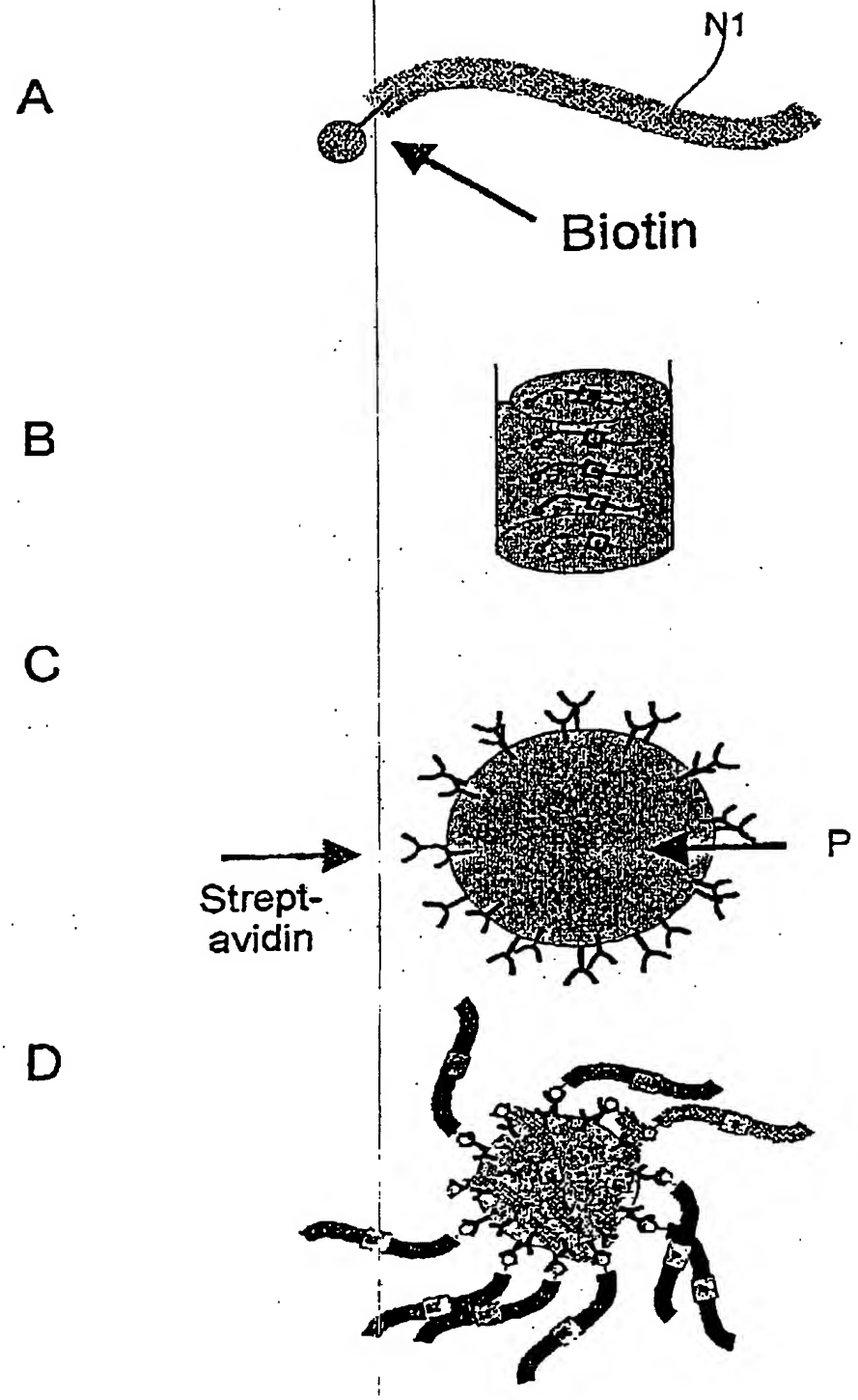


Fig. 8

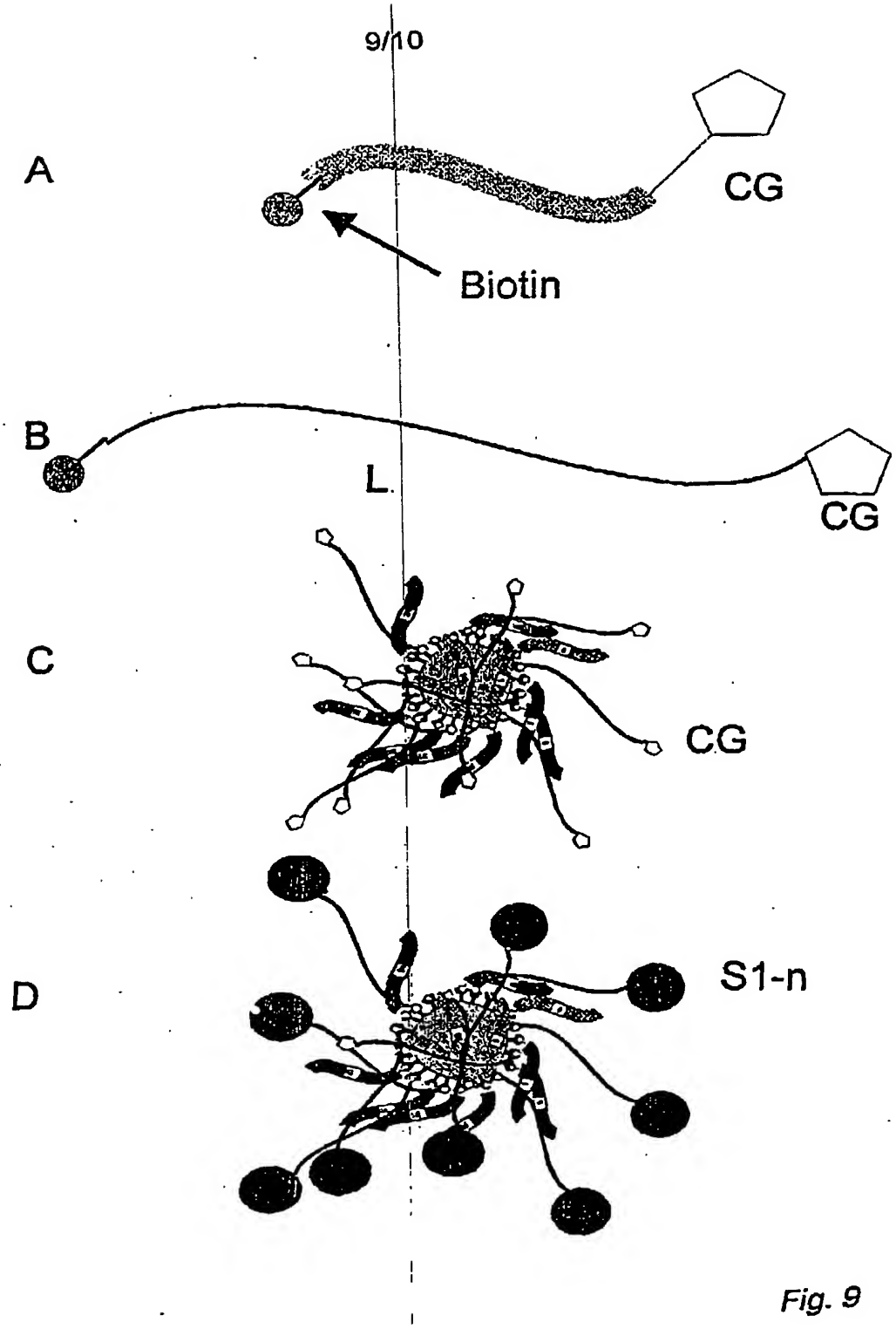


Fig. 9

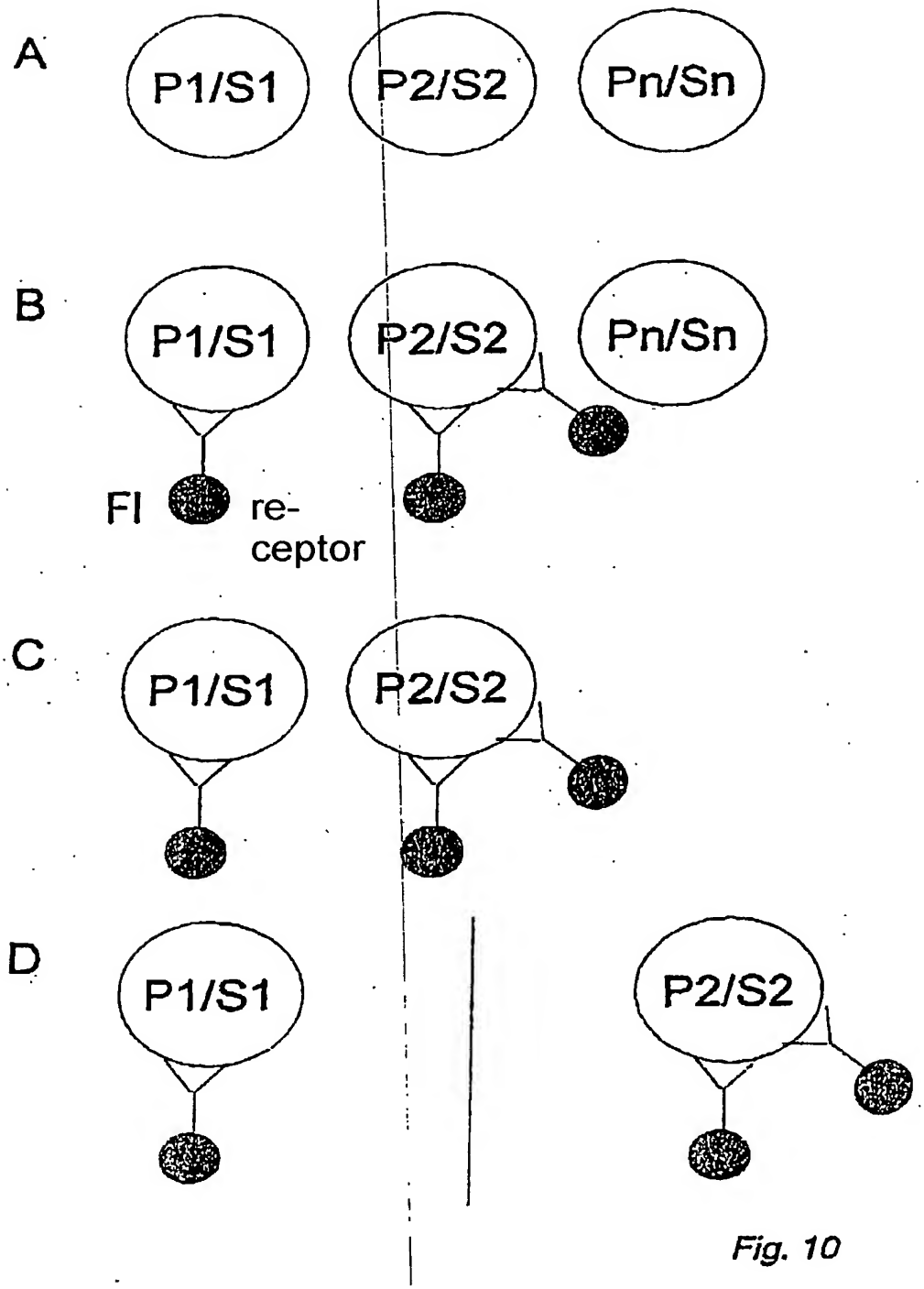

A
P1/S1
P2/S2
Pn/Sn
B
P1/S1
P2/S2
Pn/Sn
Fl re-
ceptor
C
P1/S1
P2/S2
D
P1/S1
P2/S2

Fig. 10

Attorney's Docket No.: 10848-017001
Client's Ref. No.: 412018GA-rp

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled *Method for Marking Solid, Liquid and Gaseous Substances*, the specification of which:

[] is attached hereto.
[X] was filed on January 22, 2002 as Application Serial No. 10/048,035.
[] was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
PCT	PCT/DE00/01674	May 22, 2000	[X] Yes [] No
Germany	199 34 573.2	July 22, 1999	[X] Yes [] No

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

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1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

1-00

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